REMARKS

Status of the Application

Claims 1-8, 10-12, 14-22, 24-26, 28, and 35-50 are pending in the present application.

Applicants note with appreciation the Examiner's remark that the substitute declaration is in compliance with 37 C.F.R. §1.67(a),[1] and that "the rejection for claims 1-8, 10-12, 14-22, 24-26, 28, 35 and 36 . . *is withdrawn.*"[2]

Applicants further note, and appreciate, the Examiner's recognition that "Claim 41 is free of the art of record because the art fails to teach that as little as 200-600 ES cells could successfully be used in the recited methods."[3]

Claims 38 and 47 have been cancelled and Claims 37 and 46 have been amended herein, to better define one embodiment of the invention, notwithstanding Applicants' belief that the cancelled and unamended claims would have been allowable, and without acquiescing to any of the Examiner's arguments, and without waiving the right to prosecute the unamended (or similar) claims in another application, for the purpose of furthering Applicants' business goals and expediting the patent application process in a manner consistent with the PTO's Patent Business Goals (PBG).[4] None of the amendments to the claims is related to the statutory requirements of patentability unless expressly stated so herein. No amendment made herein was intended to narrow the scope of any of the amended claims within the meaning of *Festo*.[5]

In particular, Claim 46 was amended to incorporate step d) which is recited in cancelled Claim 47. Claim 37 was amended to incorporate the limitations of claim 38.

These amendments do not introduce new matter.

The Specification and Claims 1-8, 10-12, 14-22, 24-26, 28, 35-50 have been objected to and rejected on the following grounds:

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[1] Office Action, page 2, third paragraph.

[2] (Emphasis in original) Office Action, page 5, last paragraph.

[3] Office Action, page 8, last paragraph.

[4] 65 Fed. Reg. 54603 (September 8, 2000).

[5] *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co.*, 234 F.3d 558, 56 USPQ2d 1865 (Fed. Cir. 2000) (en banc) (Nov. 29, 2000).

1. The Specification was objected to for containing references to URL;

2. Claims 42 and 45 have been objected to under 37 C.F.R. §1.75(c) for allegedly failing to further limit a prior claim;

3. Claims 1-8, 10-12, 14-22, 24-26, 28, 35-40, 42, 43, 46, 48, and 49 stand rejected under 35 U.S.C. §102(e) over Schafer *et al.*;

4. Claims 37-40, 43, 45-47, and 49 stand rejected under 35 U.S.C. §102(e) over Goodfellow; and

5. Claims 1-8, 10-12, 14-22, 24-26, 28, 35-40, and 42-50 were rejected under 35 U.S.C. §103(a) over Schafer *et al.*, Goodfellow, in view of either Kohler *et al.* or Guay-Woodford *et al.*

Applicants believe that the present amendments and the following remarks traverse the Examiner's rejection of the claims. These remarks are presented in the same order as they appear above.

1. Objection To The Specification

The Specification was objected to because it "contains references to a URL. . . . The attempt to incorporate subject matter into the patent application by reference to a hyperlink and/or other forms of browser-executable code is considered to be an improper incorporation by reference (See MPEP 608.01(p))."[6] Applicants respectfully must disagree.

The Examiner is respectfully reminded that the law regarding incorporation by reference relates to "essential material." "Essential material" is defined as:

> "that which is necessary to (1) describe the claimed invention, (2) provide an enabling disclosure of the claimed invention, or (3) describe the best mode (35 U.S.C. 112)."[7]

However, the Specification's reference to URLs was not intended to incorporate "essential material" by reference, but rather was intended to show that certain mutations, chemicals, and electromagnetic radiation were known in the art. In particular, reference to URLs was made in the Specification as follows:

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[6] Office Action, page 2, fourth paragraph.

[7] MPEP 608.01(p).

> "Cystic fibrosis was found to be associated with over 550 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [see, *e.g.*, [Zielenski and Tsui (1995) Ann. Rev. Genetics 29:777-807; Dean and Santis (1994) Hum. Genet. 93(4):364-368]. A list of the mutations associated with cystic fibrosis is available at http://www.genet.sickkids.on.ca/cftr."[8]

> "The methods of the invention are contemplated to include within their scope any agent which is capable of introducing a modification into the genome of a cell. These agents are exemplified by chemicals and electromagnetic radiation. Exemplary chemicals are described at http://dir.niehs.nih.gov/dirtb/dirrtg/chemicalsstudiedindex2.htm including, but not limited to, *N*-ethyl-*N*-nitrosurea (ENU), methylnitrosourea (MNU), procarbazine hydrochloride (PRC), triethylene melamine (TEM), acrylamide monomer (AA), chlorambucil (CHL), melphalan (MLP), cyclophosphamide (CPP), diethyl sulfate (DES), ethyl methane sulfonate (EMS), methyl methanes ulfonate (MMS), 6-mercaptopurine (6MP), mitomycin-C (MMC), procarbazine (PRC), *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG), ^3H_2O, and urethane (UR) [see, *e.g.*, Russell *et al.*, Factors affecting the nature of induced mutations, In "Biology of Mammalian Germ Cell Mutagenesis," Banbury Report 34, Cold Spring Harbor Laboratory Press (1990), pp. 271-289; Rinchik (1991) Trends in Genetics 7(1); Marker *et al.* (1997) Genetics 145:435-443]."[9]

Because the Specification's reference to URL's does not relate to "essential material," the Examiner's objection is unwarranted. Accordingly, Applicants respectfully request that the objection to the Specification be withdrawn.

2. Objection To Claims 42 And 45 Under 37 C.F.R. §1.75(c)

Claims 42 and 45 have been objected to under 37 C.F.R. §1.75(c) for allegedly "failing to further limit the subject matter of a previous claim."[10] Applicants respectfully disagree.

With respect to Claim 42, the Examiner argued that "generating a transgenic mouse with two mutations in a gene of interest . . . does not represent an additional step for

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[8] Specification, page 16, lines 7-11.

[9] Specification, page 22, lines 14-28.

[10] Office Action, page 3, first paragraph.

producing an allelic series of cells as recited in the preamble of claim 37."[11] Applicants first point out that the Examiner incorrectly states that Claim 42 requires generation of a transgenic mouse with "two" mutations in a gene of interest; rather Claim 42 recites that the mouse contains a modification "selected from" the first and second modifications. In other words, Claim 42 encompasses a mouse which contains only one (or more) of the recited modifications. Second, the Examiner states that Claim 42 "uses the cells generated by the method of claim 37."[12] This statement is an **admission** that step d) of Claim 42 further limits the scope of Claim 37 because it concedes that step d) is **additional to, and different from**, the steps of Claim 37. Applicants further note that Claim 42's step d) is indeed "an additional step for producing an allelic series" because nothing in the Specification excludes generation of an allelic series in a transgenic mouse. Rather, the Specification's following broad definition of an "allelic series of modifications" relates to the modification in the gene, rather than to the type of cell in which the gene is present:

> "The term 'allelic series' when made in reference to a gene refers to wild-type sequences of the gene. An 'allelic series of modifications' as used herein in reference to a gene refers to two or more nucleic acid sequences of the gene, where each of the two or more nucleic acid sequences of the gene contains at least one modification when compared to the wild-type sequences of the gene."[13]

Based on the above, Applicants respectfully request withdrawal of the rejection of Claim 42.

Next referring to Claim 45, the Examiner's objection is contradicted by his admission that step d) of Claim 45 is both additional to, and different from, the steps of Claim 37. In particular, the Examiner states that step d) of Claim 45 "is an *extra step* after the isolation of a modified cell."[14] This is an **admission** that Claim 45's step d) is **additional to** the steps of Claim 37. The Examiner further states that step d) of Claim 45 is "directed to a *particular method* used in step c"[15] of Claim 37. This is an **admission** that Claim 45's step d) represents

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[11] *Id.*

[12] *Id.*

[13] Specification, page 11, lines 20-24.

[14] (Emphasis added) Office Action, page 3, first paragraph.

[15] (Emphasis added) *Id.*

a preferred embodiment of Claim 37's steps, and therefore, that it is of a **different scope** from that of Claim 37's steps. Accordingly, withdrawal of the objection to Claim 45 is respectfully requested.

3. Rejection Of Claims 1-8, 10-12, 14-22, 24-26, 28, 35-40, 42, 43, 46, 48, And 49 Under 35 U.S.C. §102(e) Over Schafer *et al.*

Claims 1-8, 10-12, 14-22, 24-26, 28, 35-40, 42, 43, 46, 48, and 49 stand rejected under 35 U.S.C. §102(e) over Schafer *et al.*[16] Applicants respectfully disagree because Schafer *et al.* does not disclose all the limitations of the claims. The law is settled that:

> "Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration."[17] "[A]bsence from the reference of any claimed element negates anticipation."[18]

Schafer *et al.* discloses using chemical mutagens[19] to mutagenize whole organisms, sperm stem cells, ova, or embryonic stem (ES) cells. Following DNA analysis of a specific tissue for a mutation in a gene of interest, such as mutated ES clones in culture, the cells are transferred to the developing embryo.[20] The Examiner agreed to Applicants' characterization of Schafer *et al.*'s disclosure.[21]

Referring to Claims 1-8, 10-12, 14-22, 24-26, 28, 35, and 36, Schafer *et al.* does not disclose the **cell types** recited in step a) i), namely "fertilized egg cells and cells of 2-cell embryos." Rather, the only cell types referred to by Schafer *et al.* are "sperm stem cells or

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[16] Office Action, page 3, last paragraph.

[17] *W.L. Gore & Assoc., Inc v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303, 313 (Fed. Cir. 1983), cert. denied, 105 S. Ct. 172 (1984), citing *Soundscriber Corp. v. U.S.*, 360 F.2d 954, 960, 148 USPQ 298, 301, adopted, 149 USPQ 640 (Ct. Cl. 1966).

[18] *Rowe v. Dror*, 42 USPQ2d 1550, 1553 (Fed. Cir. 1997), citing *Kloster Speedsteel AB v. Crucible, Inc.*, 793 F.2d 1565, 1571, 230 USPQ 81, 84 (Fed. Cir. 1986).

[19] Schafer *et a*l., column 10.

[20] Schafer *et a*l., paragraph bridging column 6 and 7.

[21] Office Action, page 4, second paragraph, second sentence.

ova, . . . [and] embryonic stem (ES) cells."[22] Since Schafer omits an element of the claims, the rejection of Claims 1-8, 10-12, 14-22, 24-26, 28, 35, and 36 is improper.

Next referring to Claims 37-40, 42, and 43, Schafer *et al.* does not disclose the claims' step b)i) of treating the mouse embryonic stem cells (ESCs) with the chemical agent to produce "**at least one modification in substantially every gene**" in the mouse ESC. Claims 38-40 are distinguished from Schafer *et al.* for the additional reason that this reference does not disclose the recited **numerical values** for the modifications (*i.e.*, 70%, 85%, and 95%, respectively). For the above reasons, the rejection based on anticipation of Claims 37-40, 42, and 43 over Schafer *et al.* should be withdrawn.

As to Claims 46, 48, and 49, Schafer *et al.* does not disclose step b)'s limitation that treating the mouse ESCs with *N*-ethyl-*N*-nitrosourea results in a "**frequency of mutation in any one gene in said treated mouse embryonic stem cells is from 1/600 to 1/9,000.**" Nor does Schafer *et al.* disclose the recited step d) of detecting at least one of the first and second modifications "**using fluorescent chemical cleavage of mismatch.**" For these reasons, it is respectfully requested that the rejection of Claims 46, 48, and 49 under 35 U.S.C. §102(e) over Schafer *et al.* be withdrawn.

4. Rejection Of Claims 37-40, 43, 45-47, And 49 Under 35 U.S.C. §102(e) Over Goodfellow

Claims 37-40, 43, 45-47, and 49 stand rejected under 35 U.S.C. §102(e) over Goodfellow.[23] Applicants respectfully must disagree.

Referring first to Claims 37-40, 43, and 45, Applicants previously argued that Goodfellow does not anticipate these claims since Goodfellow does not disclose the claims' recitation of treating mouse embryonic stem cells with a chemical agent such that "**at least one modification is produced in substantially every gene in said mouse embryonic stem cells.**" The Examiner was not persuaded arguing that this limitation is disclosed by Goodfellow which "teach that the mutagenizing step should be done where about 1 mutation occurs in every 10,000-1,000 genes with the average in frequency of 1/500 and preferably

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[22] Schafer, column 6, last paragraph.

[23] Office Action, page 5, second paragraph.

1/1000-1/10,000."[24] However, the Examiner omits to consider Applicants' definition of the term "substantially every gene." The instant Specification teaches that:

> "The term 'substantially every gene' refers to the statistical probability, preferably at least about 70% probability, more preferably at least about 85% probability, and most preferably at least about 95% probability, as determined by a standard Poisson distribution, that each gene in the genome contains at least one modification."[25]

In other words, Goodfellow anticipates only if it discloses that there is **at least about 70%** probability that each gene in the genome contains at least one modification. However, this is not the case because Goodfellow discloses that the "organism has been mutagenized such that about 1 mutation occurs in every 10,000-1,000 genes,"[26] *i.e.*, **about 0.01%-0.001%**. Since there is **no overlap** in the range of mutation frequencies disclosed by Goodfellow and the claimed invention, Goodfellow does not anticipate any of Claims 37-40, 43, 45-47, and 49.

The Examiner argued that "Goodfellow *et al.* indicate that the art teaches that there may be 50-100,000 unique genes in development and 30,000 unique ESTs representing different genes."[27] However, this teaching is **irrelevant** to the claims in issue since it does not disclose that there is **at least about 70%** probability that each gene in the genome contains at least one modification following treatment with the chemical agent.

The Examiner also argued that "Goodfellow *et al.* teach that the described methods can result in 5-15 independent and different protein alterations can be generated among 10,000 organisms."[28] This teaching is also irrelevant to anticipation because it relates to alterations in **proteins** in **organisms**, rather than to modification in the recited "**gene**" in "**embryonic stem cells**."

Based on the above, Goodfellow does not anticipate Claims 37-40, 43, and 45 because it fails to disclose the limitation of "at least one modification is produced in substantially every gene in said mouse embryonic stem cells."

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[24] Office Action, page 5, last paragraph.

[25] Specification, page 14, lines 6-10.

[26] Goodfellow, column 4, lines 17-19.

[27] Office Action, page 5, last paragraph.

[28] Office Action, sentence bridging pages 5 nd 6.

Referring next to Claims 46, 47, and 49, the Examiner's rejection is moot with respect to Claim 47 in view of its cancellation herein.[29] Amended Claims 46 and 49 are not anticipated by Goodfellow since this reference does not disclose using "fluorescent chemical cleavage of mismatch" as recited in step d). Rather, Goodfellow discloses using fluorescence single-strand conformation polymorphism (SSCP).

Based on the above, Applicants respectfully request withdrawal of the rejection of Claims 37-40, 43, 45-47, and 49 under 35 U.S.C. §102(e) over Goodfellow.

5. Rejection Of Claims 1-8, 10-12, 14-22, 24-26, 28, 35-40, And 42-50 Under 35 U.S.C. §103(a) Over Schafer *et al.*, Goodfellow, Kohler *et al.*, And Guay-Woodford *et al.*

Claims 1-8, 10-12, 14-22, 24-26, 28, 35-40, and 42-50 were rejected under 35 U.S.C. §103(a) over Schafer *et al.*, Goodfellow, in view of either Kohler *et al.* or Guay-Woodford *et al.*[30] Applicants respectfully traverse because a *prima facie* case of obviousness is not established.

A *prima facie* case of obviousness requires the Examiner to cite to a combination of references which (a) discloses the elements of the claimed invention, (b) suggests or motivates one of skill in the art to modify their teachings to yield the claimed invention, **and** (c) provides a reasonable expectation of success should the claimed invention be carried out. Failure to establish **any** one of these requirements precludes a finding of a *prima facie* case of obviousness and, without more, entitles Applicants to withdrawal of the rejection.[31] Applicants urge that the Examiner has failed to establish not just one, but **all three** requirements as discussed below.

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[29] Claim 47 has been cancelled notwithstanding Applicants' belief that the cancelled claim would have been allowable, and without acquiescing to any of the Examiner's arguments, and without waiving the right to prosecute the unamended (or similar) claims in another application, for the purpose of furthering Applicants' business goals and expediting the patent application process in a manner consistent with the PTO's Patent Business Goals (PBG).

[30] Office Action, page 7, first paragraph.

[31] See, *e.g.*, *Northern Telecom Inc. v. Datapoint Corp.*, 15 USPQ2d 1321, 1323 (Fed. Cir. 1990); *In re Dow Chemical Co.*, 837 F.2d 469, 5 USPQ2d 1529 (Fed. Cir. 1988).

A. The Combined References Do Not Teach All The Elements Of The Rejected Claims

It is axiomatic for establishing a *prima facie* case of obviousness that "all the claim limitations must be taught or suggested by the prior art."[32] This has not been established.

With respect to Claims 1-8, 10-12, 14-22, 24-26, 28, 35, and 36, none of the references discloses or suggests the **cell types** recited in step a) i), namely "fertilized egg cells and cells of 2-cell embryos."

With regards to Claims 37-40, and 42-45 the combined references are silent on the limitation of "**at least one modification is produced in substantially every gene in said mouse embryonic stem cells.**"

As to Claims 37-40, 42, and 43, and 45, none of the references discloses or suggests the recited step b)i) of treating the mouse embryonic stem cells (ESCs) with the chemical agent to produce "**at least one modification in substantially every gene**" in the mouse ESC. In addition, the references do not teach Claims 38-40's recitation of the **numerical values** for the modifications (*i.e.*, 70%, 85%, and 95%, respectively) in the gene of interest. Moreover, with respect to Claims 45, the references do not discloses or suggest using the recited "**fluorescent chemical cleavage of mismatch.**"

Referring to Claims 46-49, none of the references discloses step d) of detecting at least one of the first and second modifications "**using fluorescent chemical cleavage of mismatch.**"

Since the combined references do not teach or suggest at least one limitation of the claims, a *prima facie* case of obviousness cannot stand.

B. The Combined References Do Not Provide Motivation To Practice The Claimed Methods

The threshold requirement for a *prima facie* case of obviousness is whether a person skilled in the art would be **motivated** to modify the references to arrive at the **claimed invention**.[33] In particular,

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[32] MPEP 2143.03, citing *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

[33] *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598-99 (Fed. Cir. 1988) and *In re Jones*, 21 USPQ2d 1941, 1943 (Fed. Cir. 1992).

> "the examiner must show *reasons* that the skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would *select* the elements from the cited prior art references for combination in the manner claimed."[34] Evidence of a suggestion, teaching, or motivation to modify prior art references "must be *clear and particular*."[35]

The Examiner's only argument with respect to "motivation" is that there is motivation "to pick p53 and PKD genes as genes of interest because of their implicated roles in human diseases."[36] Importantly however, this argument is **irrelevant** to Claims 1-8, 10-12, 14-22, 24-26, 28, 35-40, and 42, 43, 45-49 because none of these claims recites either p53 or PKD genes.

The Examiner is respectfully reminded that the **claimed invention** in Claims 1-8, 10-12, 14-22, 24-26, 28, 35, and 36, recites using "fertilized egg cells and cells of 2-cell embryos." The Examiner did not allude to any teaching in the combined references which provides a motivation to use these target cells. Accordingly, a *prima facie* case of obviousness cannot be established with respect to Claims 1-8, 10-12, 14-22, 24-26, 28, 35, and 36.

The Examiner is also respectfully reminded that the **claimed invention** of Claims 37-40, 42, and 43, and 45, recites treating the mouse embryonic stem cells (ESCs) with the chemical agent to produce "**at least one modification in substantially every gene**" in the mouse ESC [step b)i)]. However, the Examiner did not advance any reasons to provide motivation to arrive at this limitation. Moreover, with respect to Claims 38-40, the Examiner is silent on how the references provide motivation to arrive at the recited **numerical values** for the modifications (*i.e.*, 70%, 85%, and 95%, respectively) in the gene of interest. In addition, the Examiner did not advance any reasons to provide motivation with respect to Claim 45-49's recitation of using "**fluorescent chemical cleavage of mismatch**."

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[34] (Emphasis added) *In re Rouffet*, 47 USPQ2d 1453 (Fed. Cir. 1998); *Robotic Vision Systems Inc. v. View Engineering Inc.*, 51 USPQ2d 1948 (Fed. Cir. 1999).

[35] (Emphasis added) *In re Dembiczak*, 175 F.3d 994, 50 USPQ2d 1614 (Fed. Cir. 1999), *citing C.R. Bard*, 157 F.3d 1340 at 1352, 48 USPQ2d at 1232.

[36] Office Action, page 8, first paragraph.

For the above reasons, motivation to modify the combined references to arrive at the claimed methods has not been established. Accordingly, a *prima facie* case of obviousness must fail.

C. A Reasonable Expectation of Success In Practicing The Recited Methods Is Not Established

A fundamental requisite of establishing a *prima facie* case of obviousness is that there is a reasonable expectation of success in making and using the recited sequences.

> "[T]he reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure."[37]

This has not been shown. The Examiner argued that there "would have been a reasonable expectation of success to target p53 and PKD as genes of interest given the *successful results* of Schafer *et al.* and Goodfellow *et al.* for several other genes of interest, and the teachings of both Kohler *et al.* and Guay-Woodford *et al.* that specific mutations *can be made* and already exist and result in detectable phenotypic changes."[38] The problem with this argument is that it does not explain how a reasonable expectation of success can be extrapolated from (a) Schafer *et al.*'s methods which use a different target cell for the chemical treatment than the recited target cells, (b) the experimental conditions of Schafer *et al.* and Goodfellow to the recited production of "at least one modification in substantially every gene," and to the recited numerical values (*i.e.*, 70%, 85%, and 95%) for the modifications, and (c) Goodfellow's different fluorescent SSCP method of detection as compared to the recited method of "fluorescent chemical cleavage of mismatch."

Since the Examiner has not demonstrated (as he must) that the reasonable expectation of success is founded in the prior art rather than in Applicants' disclosure, the third prong of a *prima facie* case of obviousness is defective.

Because, not one, but each of the three elements of a *prima facie* case of obviousness is lacking, a *prima facie* case of obviousness cannot be established. It is therefore respectfully

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[37] *In re Dow Chemical Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988) as cited in *In re Vaeck*, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).

[38] (Emphasis added) Office Action, page 8, first paragraph.

requested that the rejection of Claims 1-8, 10-12, 14-22, 24-26, 28, 35-40, and 42-50 under 35 U.S.C. §103(a) for alleged obviousness be withdrawn.

Conclusion

All grounds of rejection and objection of the Office Action of January 16, 2002 having been addressed, reconsideration of the application is respectfully requested. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicants encourage the Examiner to call the undersigned collect at (617) 252-3353.

Signed on behalf of:

Dated: July 16, 2002

Maha A. Hamdan
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APPENDIX I

MARKED-UP VERSION OF REWRITTEN, ADDED, AND/OR CANCELLED CLAIMS

The following is a marked-up version of the claims pursuant to 37 C.F.R. §1.121 (c)(1)(ii) with instructions and markings showing changes made herein to the previous version of record of the specification and claims. Brackets denote deleted text, and underlining denotes added text.

IN THE CLAIMS

Cancel Claims 38 and 47.

Amend Claims 37 and 46 as follows:

37. (Once Amended) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;

ii) a chemical agent capable of producing at least one modification in said gene of interest;

b) treating said mouse embryonic stem cells with said chemical agent under conditions such that (i) at least one modification in substantially every gene in said mouse embryonic stem cells is produced, and (ii) a mixture of embryonic stem cells comprising said gene of interest is produced, said mixture of embryonic stem cells comprising embryonic stem cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest, wherein said treating is under conditions such that at least one modification in at least 70% of the genes in said mouse embryonic stem cells is produced; and

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells.

46. (Once Amended) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;

ii) *N*-ethyl-*N*-nitrosourea;

b) treating said mouse embryonic stem cells with said *N*-ethyl-*N*-nitrosourea to produce treated mouse embryonic stem cells comprising a mixture of embryonic stem cells, said mixture comprising embryonic stem cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest, wherein the treatment is under conditions such that the frequency of mutation in any one gene in said treated mouse embryonic stem cells is from 1/600 to 1/9,000; [and]

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells; and

d) detecting at least one of said first and second modification in said gene of interest using fluorescent chemical cleavage of mismatch.